Inward Rectifier K Channels

2326-Pos Board B312
Engineered pH-Dependence at the Kir6.2 Helix Bundle Crossing
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The hallmark functional property of K_{ATP} (ATP-sensitive potassium) channels is inhibition by intracellular ATP, which binds to a well-defined binding site on Kir6.x subunits and stabilizes the closed conformation of a gate in the channel pore. Numerous inwardly-rectifying potassium (Kir) channels possess an aromatic residue in the 'helix bundle crossing' region, forming the narrowest pore constriction in crystal structures of Kir channels, indicating an important role in channel gating. We have identified a remarkable phenotype of mutant channels carrying a glutamate at this position (F168E). Despite the structural prediction of four glutamates in close proximity, F168E channels are predominantly closed at physiological pH. However, intracellular alkalinization causes rapid and reversible channel activation. These findings suggest that F168E glutamates are uncharged at physiological pH but become deprotonated with a pKa~9, resulting in opening due to mutual repulsion of multiple nearby glutamate sidechains. The K-channel pore scaffold likely brings these glutamates into close proximity, stabilizing the protonated (uncharged) form of the glutamate sidechain, and resulting in a dramatic pKa shift relative to free glutamate. Only at more alkaline pH do the glutamates deprotonate, with their mutual repulsion driving channel opening. Consistent with a role in ATP-mediated channel closure, alkalinization also affects channel sensitivity to ATP. Taken together, these findings demonstrate an engineered (not intrinsic) mechanism of channel gating by pH, and suggest that ATP-mediated gating of Kir6.2 involves conformational rearrangement of the bundle crossing region.

2327-Pos Board B313
The Kir5.1 Potassium Channel is an Important Determinant of Neuronal PCO2/pH Sensitivity
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The molecular identity of ion channels which respond to PCO2/pH in the brain is still unclear. Heteromeric Kir4.1/Kir5.1 channels are highly sensitive to inhibition by intracellular pH and are widely expressed in several brainstem nuclei involved in cardiorespiratory control, including the locus coeruleus (LC). This has therefore led to a proposed role for these channels in neuronal CO2-chemosensitivity. In order to examine this we generated mutant mice lacking the Kir5.1 (Kcnj16) gene. We show that whilst LC neurons from Kcnj16 (+/+) mice rapidly respond to cytoplasmic alkalinization and acidification, those from Kcnj16(−/−) mice display a dramatically reduced and delayed response. These results identify Kir5.1 as an important determinant of PCO2/pH sensitivity in locus coeruleus neurons and suggest that Kir5.1 is involved in the response to hypercapnic acidosis.

2328-Pos Board B314
Flexibility Between the Linker of the CD and G-Loops Determines the Gating Dynamics of Hte Kir2.1 Channel
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Inwardly rectifying K^+ (Kir) channels are important regulators of the resting membrane potential and cell excitability. The activity of Kir channels is critically dependent on the integrity of channel-PIP_2 (phosphatidylinositol 4, 5-bisphosphate).
Molecular Dynamics (MD) simulations predict interactions of specific residues with PIP_2. Mutagenesis data are in good agreement with the theoretical predictions.Here we measure Kir channel gating kinetics regulated by endogenous and exogenous PIP_2. Our data show that one Kir2.1 channel mutation, V223L, which is not predicted to interact with PIP_2 directly altered the PIP_2 apparent affinity by approximately 3-fold (the EC_{50} was 2.75 ± 0.12 μM for WT channel and 8.57 ± 0.76μM for the V223L mutation). V223 is localized in the CD-loop that has been implicated in the control of PIP_2-dependent gating. V223 is predicted to interact with M301 in the G-loop, thus providing a coupling mechanism between the CD and G-loops. Unlike mutations of residues that are predicted to affect directly or indirectly channel-PIP_2 interactions, V223L accelerated both the rates of inhibition (PIP_2 depletion) and reactivation (PIP_2 replenishment). Based on MD simulation results, we show that the V223L mutation enhances the flexibility of the amino acids from 270 to 290 which are located between the CD and G loops. Increasing the viscosity of the bathing solution reduces the dynamics of proteins, and consistent with the MD simulation results also in slowing down the kinetics of V223L inhibition and reactivation. Our results offer for the first time a link between the CD and G-loops in transducing the PIP_2 effect on gating the Kir2.1 channel.

2329-Pos Board B315
G protein Gating of a Kir3.1-Prokaryotic Kir Channel Chimera Functionally Reconstituted in Planar Lipid Bilayers
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Functional reconstitution of the purified chimeric Kir3.1 channel constituting the cytosolic domain of mammalian Kir3.1 and the transmembrane region of a prokaryotic KirBac1.3 was achieved in lipid bilayers by addition of PIP_2 from the intracellular side. Chimera had the typical traits of an inwardly rectifying potassium channel (Kir); PIP_2- and Mg^{2+}-dependence. Additionally, the chimera exhibited typical sidedness of a Kir3 channel: channel activity could be blocked by external (*Trans*) Tertiapin Q and by internal (*Cis*) poly Lysine and PIP_2 antibody. Chimeric channels could also be stimulated by internal application of ethanol. Either of the G-protein subunits Gα-GDP or Gβγ alone or together and in either order of application inhibited PIP_2-activated channel currents. In contrast, addition of GTPγS, following inhibition by both Gα-GDP and Gβγ, caused channel stimulation. Alternatively, addition of GTPγS following inhibition by Gα-GDP had no effect but further addition of Gβγ caused channel stimulation. Thus, gating of the chimeric channel required both activated forms of α, (α-GTPγS) and βγ subunits of G-proteins. This result is reminiscent of the requirement of both active $G\alpha_s$ and Gβγ subunits for activation of Gβγ-sensitive isoforms of adenylyl cyclase. Mammalian Kir3 channels expressed in native or heterologous systems do not exhibit a requirement for activated G-protein subunits and this interesting difference ought to addressed in future. A 3D reconstruction of the chimera by single particle electron microscopy indicated a structure consistent with the crystal structure. Our results confirm that the chimera is a reasonable structural and functional model for regulation of effectors by G protein subunits. Moreover our ability to reconstitute modulation of channel currents by G protein subunits in planar lipid bilayers offers a unique opportunity to dissect precise roles for each component of the signaling complex.

2330-Pos Board B316
Cholesterol Binding Regulates Prokaryotic Kir Channels
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Cholesterol is a major regulator of a variety of ion channels but the mechanisms underlying cholesterol sensitivity of ion channels are still poorly understood. The key question is whether cholesterol regulates ion channels by specific binding to the channel protein or by altering the physical environment of lipid bilayer. In this study, we provide the first direct evidence that cholesterol specifically binds to prokaryotic Kir channels, KirBac1.1, and that cholesterol binding is essential for its regulatory effect. Specifically, we show that cholesterol is eluted together with the KirBac1.1 protein when separated on an affinity column and that the amount of bound cholesterol is proportional to the amount of the protein. We also show that cholesterol binding to KirBac1.1 is saturable with a Km of 390 μM. Moreover, there is clear competition between radioactive and non-radioactive cholesterol for the binding site. There is no competition, however, between cholesterol and 5-Androsten 3β-17[]β-diol, a sterol that we showed previously to have no effect on KirBac1.1 function. Finally, we show that cholesterol-KirBac1.1 binding is significantly inhibited by trifluoperazine, known to inhibit cholesterol binding to other proteins, and that inhibition of cholesterol-KirBac1.1 binding results in full recovery of the channel function. We conclude, therefore, that cholesterol-induced suppression of KirBac1.1 activity is mediated by direct interaction between cholesterol and the channel protein.

2331-Pos Board B317
Drug-Induced hERG Current Enhancement is Modulated by Extracellular Proton and Potassium
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Acid-base disturbances and hypokalemia or hyperkalemia are frequently encountered in a variety of clinical scenarios. Extracellular proton and potassium concentrations are important modulators of hERG channel functions and biophysical properties such as deactivation and inactivation. Slowing deactivation and inactivation kinetics of hERG channel are two important components for drug-induced hERG current enhancement. This study examined if drug-induced hERG current enhancement is affected by acid-base and electrolyte aberrations at physiological temperature (37° C). Whole-cell voltage clamp technique was used to measure hERG current (step-ramp protocol: initial 1-sec depolarization step to 0 mV from −80 mV (V_H) followed by a 2-sec repolarization ramp back to V_H) and a typical hERG activator A-935142 was used as a tool drug. Drug-induced hERG current enhancement was potentiated by